Surgical Manipulation of the Gut Elicits an Intestinal Muscularis Inflammatory Response Resulting in Postsurgical Ileus

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Objective

To investigate the pathophysiologic mechanisms that lead to ileus after abdominal surgery.

Summary Background Data

The common supposition is that more invasive operations are associated with a more extensive ileus. The cellular mechanisms of postsurgical ileus remain elusive, and few studies have addressed the mechanisms.

Methods

Rats were subjected to incremental degrees of surgical manipulation: laparotomy, eventration, "running," and compression of the bowel. On postsurgical days 1 and 7, muscularis infiltrates were characterized immunohistochemically. Circular muscle activity was assessed using mechanical and intracellular recording techniques *in vitro*.

Results

Surgical manipulation caused an increase in resident phagocytes that stained for the activation marker lymphocyte function–associated antigen (LFA-1). Incremental degrees of manipulation also caused a progressive increase in neutrophil infiltration and a decrease in bethanechol-stimulated contractions. Compression also caused an increase in other leukocytes: macrophages, monocytes, dendritic cells, T cells, natural killer cells, and mast cells.

Conclusion

The data support the hypothesis that the degree of gut paralysis to cholinergic stimulation is directly proportional to the degree of trauma, the activation of resident gut muscularis phagocytes, and the extent of cellular infiltration. Therefore, postsurgical ileus may be a result of an inflammatory response to minimal trauma in which the resident macrophages, activated by physical forces, set an inflammatory response into motion, leading to muscle dysfunction.

Postsurgical ileus is a persistent feature of abdominal surgery that can lead to significant morbidity and mortality rates.[1,2] Ileus is generally accepted as a normal response to abdominal surgery. Besides causing significant patient discomfort (respiratory compromise, abdominal distention, nausea, emesis, and pain) and more serious problems (*e.g.*, acute gastric dilatation, aspiration), ileus prolongs the hospital stay, delays early enteral nutrition, and increases medical costs. Despite the widespread presence of this problem, little is known about the underlying pathogenesis.

Multiple causes of postsurgical ileus have been suggested, including the involvement of sympathetic reflexes, inhibitory humoral agents, norepinephrine release from the bowel wall, effects of anesthetic agents, and inflammation.[2,3] The results from *in vivo* studies of the extent, location, and duration of postsurgical ileus have been contradictory.[2,4–6] Since the introduction of laparoscopic techniques, reports have been published indicating that minimally invasive surgery is accompanied by a much shorter period of postsurgical ileus, suggesting that surgical manipulation *per se* may play an important role in its causation.[7–10] However, controversy continues.[11,12]

The cellular mechanisms of postsurgical ileus have received little scientific investigation. Most investigations have focused primarily on intestinal transit times[13–15] or *in vivo* recording of alterations in the migrating motor complex.[4,6,16] Although myoelectric activity has been demonstrated to return to normal within 24 hours after the limited

insult of a laparotomy,[17] it appears not to be absolutely predictive of "clinical" recovery.[9,18,19] Interpretation of these studies may be confounded by the use of various species and different surgically induced insults.

In 1993, Taché et al.[20] published an elegant hypothesis of at least one type of stress-induced gastric inhibition. In their scenario, psychological or cecal manipulation causes the central release of corticotropin-releasing factor in the paraventricular nucleus of the hypothalamus and the dorsal vagal complex. This central neural activity is hypothesized to stimulate an efferent inhibitory motor pathway. In contrast to gastric inhibition, colonic transit is paradoxically increased by the intracisternal administration of corticotropin-releasing factor. An increase in colonic motility is consistent with the clinical observation of increased colonic activity during psychological stress. However, postsurgical ileus is characterized by a decrease in intestinal myoelectric activity and transit.

Many investigators have sought a peripheral mechanism for the cause of postsurgical ileus, with minimal success. Few studies have addressed the contribution of local factors, such as inflammation, that could lead to surgically induced ileus.[2] Recently, we have observed the presence of a dense network of macrophages resident in the intestinal muscularis. These normally quiescent macrophages were observed to upregulate their expression of the membrane marker for "activation" lymphocyte function–associated antigen (LFA-1) in response to in vitro surgical tissue dissection.[21] Also, endotoxin administered systemically appears to activate these resident leukocytes,[22] and the activation is accompanied by the appearance of paralytic ileus. We hypothesize that commonly performed surgical procedures of the intestine trigger the activation of the muscularis macrophage network and initiate the recruitment of leukocytes, and that these cellular events cause a period of postsurgical dysmotility. This study was designed to determine the cellular and functional consequences of various degrees of mild surgical manipulation on rat jejunal muscularis externa.

MATERIALS AND METHODS

Animals

ACI male rats (200 to 250 g) were obtained from Harlan-Sprague-Dawley (Indianapolis, IN). The protocol was approved by the University of Pittsburgh Institutional Animal Care and Use Committee. Rats were housed in a pathogen-free facility that is accredited by the American Association for Accreditation of Laboratory Animal Care and complies with the requirements of humane animal care as stipulated by the United States Department of Agriculture and the Department of Health and Human Services. They were maintained on a 12-hour light/dark cycle and given commercially available rat chow and tap water *ad libitum.*

Experimental Groups and Surgical Procedures

The small bowel was subjected to incremental degrees of gentle surgical manipulation. The rats were anesthetized with methoxyflurane inhalation, and a midline abdominal incision was made. The surgical procedures were performed under sterile conditions: when the intestine was manipulated, only instruments were used; it was never touched directly. During and after the procedure, the animal was positioned under a heating lamp, watched until it recovered from the anesthesia, and returned to a cage.

Four groups of five animals each were established, each representing a different level of intestinal manipulation. Group I underwent a midline laparotomy and had the abdomen left open for 10 minutes under a moist gauze tent. Group II had the small intestine eventrated to the left and kept on a moist cotton gauze for 10 minutes. Group III had the entire bowel gently inspected along its whole length using moist cotton applicators; this group was designed to simulate the usual "running" of the bowel in the clinical situation. Group IV had the bowel "run" so that the small bowel contents were made to pass into the cecum by an aboral compression of the gut lumen, using the simultaneous rolling action of two moist cotton applicators. In this group, the bowel was eventrated to the left and positioned on moist gauze; with two moist cotton applicators, the entire small bowel luminal contents were evacuated by simultaneously rolling the applicators toward the cecum. In all groups, the incision was closed using two-layer continuous sutures. All animals were killed at 24 hours.

Two additional compression groups were also studied. These animals underwent the same procedure as described for group IV but were killed either immediately after the procedure (group V) or on postoperative day (POD) 7. Age-matched rats not undergoing anesthesia or laparotomy served as controls.

Small Bowel Preparation

Animals were anesthetized with methoxyflurane for a second time when they were killed. The abdominal wound was reopened and the inferior vena cava and abdominal aorta were cannulated. The abdominal aorta was clamped below the diaphragm and the superior mesenteric artery was flushed with 3 ml of cold (4°C) Ringer's solution (Baxter, Deerfield, IL). This flush was used to remove nonadhered and nonextravasated blood cells from the vasculature. The entire small bowel was then removed and placed in cold preoxygenated Krebs-Ringer buffer (KRB). The functional studies described below were carried out immediately on intestinal segments consistently taken from the middle jejunum. Histochemical and immunohistochemical studies were carried out on bowel specimens consistently obtained from the distal jejunum.

Functional Studies

Mechanical activity was measured as previously described.[22] Briefly, a segment of midjejunum was pinned in a dissecting dish containing iced, preoxygenated KRB and opened along the mesentery. Full-thickness strips (1×10 mm) were cut parallel to the circular muscle layer and suspended in standard horizontal mechanical organ chambers, which were continuously perfused with preoxygenated KRB solution maintained at 37°C. One end of the strips was tied to a fixed post and the other attached to an isometric force transducer (WPI, Sarasota, FL). In the organ chamber, each strip was allowed to equilibrate for 1 hour. Strips were then incrementally stretched to L_0 (length at which maximal contraction occurs). Dose-response curves were generated by exposing the muscles to increasing concentrations of the muscarinic agonist bethanechol (0.1 to 300 μM) for 10 minutes, followed by a 15-minute wash period. Contractile activity was calculated as $g/mm^2/sec$ by converting the weight and length of the strip to square millimeters of tissue.

Spontaneous intracellular electrical activity and neurally mediated circular muscle postsynaptic membrane responses elicited with electrical field stimulations were also recorded from control and compression-treated muscle strips. Muscle strips for these recordings were cut (1×10 mm) parallel to the circular muscle layer and were pinned in cross-section into an electrophysiologic recording chamber. Cross-sectional pinning of the muscle allowed the placement of the recording microelectrode into circular muscle cells located halfway through the thickness of the muscle layer.[23] Activity was recorded after an equilibration period of 2 hours. Single circular smooth muscle cells were impaled with 20 to 50 ΩM glass microelectrodes filled with 3M KCl. Intracellular recordings were accepted when a sharp voltage drop of greater than 50 mV was observed, a stable resting membrane potential persisted for 5 minutes, and spontaneous electrical activity was generated. Impalements were held for up to 2 hours without a significant change in the resting membrane potential. Intracellular potentials were recorded by a high-impedance amplifier and displayed on an oscilloscope (Tektronix, Heerenveen, The Netherlands). Membrane potentials were recorded on VHS tape (Sony, Park Ridge, NJ) after pulse code modulation (Instrutec, Elmont, NY) for off-line analysis (Acknowledge A/D program, Biopac Systems, Santa Barbara, CA).

Tetrodotoxin-sensitive electrical field–stimulated postsynaptic membrane responses were elicited by passing current across two platinum wires running parallel along the side of each muscle strip (pulse duration 0.8 milliseconds, frequencies 1 to 20 Hertz, stimulus durations 1 to 100 seconds, voltage 150 V). Square wave voltage pulses across the platinum wires were generated by an S11 voltage stimulator connected to an SIU-5 stimulus isolation unit (Grass Instruments, Quincy, MA).

Morphologic Studies

Segments of midjejunum were fixed in 10% formalin and embedded in paraffin. Jejunal cross-sections (5 μm) were stained with hematoxylin and eosin for morphologic examination. Specimens were evaluated microscopically in a blinded fashion by two investigators (AJB and JCK) and graded for the determination of bowel wall tissue damage. The severity of injury was graded as follows:

- Grade 0: no specific pathologic changes; normal visualization of gut wall architecture, including villi, crypts, lamina propria, and muscularis externa
- Grade 1: mild damage; denudation of villi epithelium, otherwise normal structure
- Grade 2: moderate damage; loss of villus height and epithelial sloughing with evidence of congestion, hemorrhage, and inflammation in the mucosa, but no changes in the submucosa or muscularis externa
- Grade 3: extensive damage; loss of a large number of villi including denudation, sloughing, and the presence of granulomatous tissue, with the damage localized to the submucosa and muscularis
- Grade 4: severe damage and necrosis; inflammation and necrosis throughout the thickness of the intestinal wall.

Histochemistry and Immunohistochemistry

Midjejunal segments were cut from the bowel and immersed in KRB in a Sylgard-filled glass dish in an ice bath at 4°C, as previously described.[21] Each standardized 5-cm opened segment of jejunum was fixed in 100% ethanol for 10 minutes or in 4% paraformaldehyde for 10 minutes. A segment was washed twice in KRB, and the mucosa and submucosa were stripped off under microscopic observation (Wild M-8, Heerbrugg, Switzerland). The mucosa-free muscularis whole mounts were used for staining procedures. The following histochemical stains were used: myeloperoxidase (MPO) for polymorphonuclear neutrophils (PMNs), NADPH diaphorase for nitric oxide (NO)–containing neurons,[24] and FITC-labeled avidin for mast cells. All histochemically stained whole mounts were coverslipped and counted. Muscularis whole mounts were also used for immunohistochemical analysis of the jejunal muscularis. Each whole mount was incubated overnight at 4°C in the primary antibody, followed by three 5-minute washes in 0.05 M phosphate-buffered saline (PBS). The specimens were then incubated in the appropriate secondary antibody at 4°C for 4 hours and washed three times for 5 minutes each in PBS. The following primary and secondary antibodies were used: ED1 (1:100), monocytes; ED2 (1:100), resident macrophages; OX19 (1:100), T cells; LFA-1 (1:50), CD11/CD18; OX76 (1:5), MHC class II; OX62 (pure), dendritic cells; HNK-1 (1:2), natural killer (NK) cells; OX33 (1:10), B cells; and tryptase (1:250), mast cells. Antibodies were used for whole-mount immunohistochem-

istry at the described concentrations, which may vary extensively in frozen or paraffin-embedded cross-sections. Specificity is given in accordance with the individual manufacturer's information.

Whole mounts were cover-slipped and inspected by light or fluorescent microscopy after staining (Nikon FXA, Fryer, Huntley, IL). Leukocytes were counted in five randomly chosen areas in each specimen at a magnification of ×200. Indirect immunoperoxidase staining was carried out on whole mounts using an avidin—biotin complex staining kit (ABC Elite, Vector, Burlingame, CA), rinsed in dH_2O, and mounted in Gel-Mount (Biomeda, Foster City, CA). Formalin-fixed and paraffin-embedded segments of midjejunum were cut at 5 μm and also used for histochemistry and immunohistochemistry.

Myeloperoxidase Assay

The isolated mucosa and muscularis were measured for MPO activity. The harvested entire small bowel was cut into 5-cm segments, which were pinned down in a dissection dish. The mesentery was removed and the segment was slid onto a glass rod. The muscularis was incised carefully and stripped off the mucosa circumferentially with a moist cotton applicator. The results of this procedure were histologically confirmed. MPO was measured photometrically using the method described by Allan et al.[25] MPO was calculated as mUnits/g tissue, with one unit of MPO degrading 1 μmol peroxide/minute at 25°C.

Data Analysis

Data were compiled as mean ± standard error of the mean. Statistical analysis was performed using the unpaired Student's t test. Statistical significance was assumed at p < 0.05.

Drugs and Solutions

A standard oxygenated KRB was used. KRB constituents and bethanechol were obtained from Sigma Chemical (St. Louis, MO). The antibodies ED1, ED2, OX19, OX76, OX62, and LFA-1 were purchased from Serotec, Harlan Bioproducts for Science (Indianapolis, IN). Mast cells were stained with an antitryptase antibody obtained from Chemicon International (Temecula, CA). RPE-conjugated goat–antimouse secondary antibody was purchased from DAKO (Carpinteria, CA). Antibodies were diluted in 0.05M PBS containing 0.2% bovine serum albumin (Sigma), 100 U/ml penicillin G, and 100 μg/ml streptomycin (Boehringer Mannheim, Indianapolis, IN). HNK-1 was a kind gift from Dr. Willi Halfter (University of Pittsburgh).
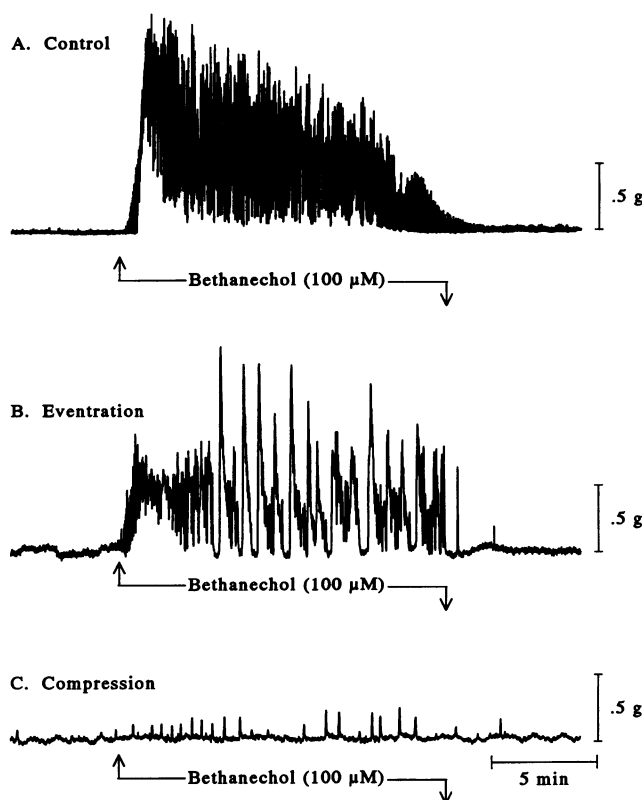

Figure 1. Organ bath recorded mechanical traces of bethanechol-stimulated (100 μM) circular muscle strips. Panel A shows the trace in a control animal, Panel B after eventration, and Panel C after compression, both on postoperative day 1.

RESULTS

General Observations

The surgical procedures resulted in no deaths or major surgical complications (including hemorrhage, peritonitis, or perforation). All animals appeared healthy, with early postsurgical fluid intake and normal behavior.

Functional Studies

In vitro measurement of intestinal smooth muscle contractile activity after surgery has not been previously investigated. We hypothesized that the inflammatory cells in the intestinal muscularis would result in changes in the function of the smooth muscle. Control jejunal muscle strips spontaneously exhibited regular monophasic contractions with a frequency of 28 ± 2.5 events/minute (n = 9). Stimulation of the control muscles with the muscarinic agonist bethanechol resulted in the generation of large and relatively regular phasic contractions overlying a tonic contractile event. Contractility decreased in all manipulated groups compared with controls. Figure 1 depicts the decrease in contractile force of mechanical traces recorded from an organ bath after eventration and compression compared with controls. Significant changes were seen in the running (group III) and
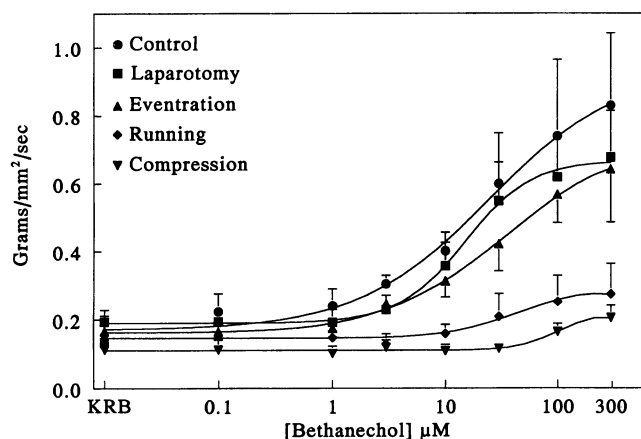
Figure 2. Bethanechol-stimulated dose-response curves of circular muscle contractile activity generated from jejunal muscle after increasing degrees of surgical manipulation. Data are expressed as mean ± SEM. N = 5 to 7.

Table 1. HISTOLOGY / MORPHOLOGIC DAMAGE

Group	Manipulation	Time	Grade
Controls	None	Immediately	0 ± 0
I	Laparotomy	24 hours	0.3 ± 0.18
II	Eventration	24 hours	1.6 ± 0.24†
III	Running	24 hours	2.0 ± 0.32†
IV	Compression	24 hours	2.9 ± 0.23†
V	Compression	Immediately	1.0 ± 0.41†
VI	Compression	7 days	0.7 ± 0.23*

Formalin fixed and paraffin-embedded cross-sections were stained with H&E and evaluated microscopically in a blinded fashion by two investigators. Histologic damage was graded as defined in the Method section. Data are expressed as mean ± SEM. N = 5–10.
* $p < 0.05$ vs. controls.
† $p < 0.05$ vs. Group I.
H&E = hematoxylin-eosin.

compression (group IV) groups (controls, 0.83 ± 0.212; group III, 0.27 ± 0.089; group IV, 0.20 ± 0.036 g/mm²/sec at 300 μM bethanechol) (Fig. 2). Contractile activity measured immediately after compression showed a significant decrease (group V, 0.20 ± 0.043) compared with controls. This immediate decrease in circular muscle function would appear not to be the result of surgically induced muscle
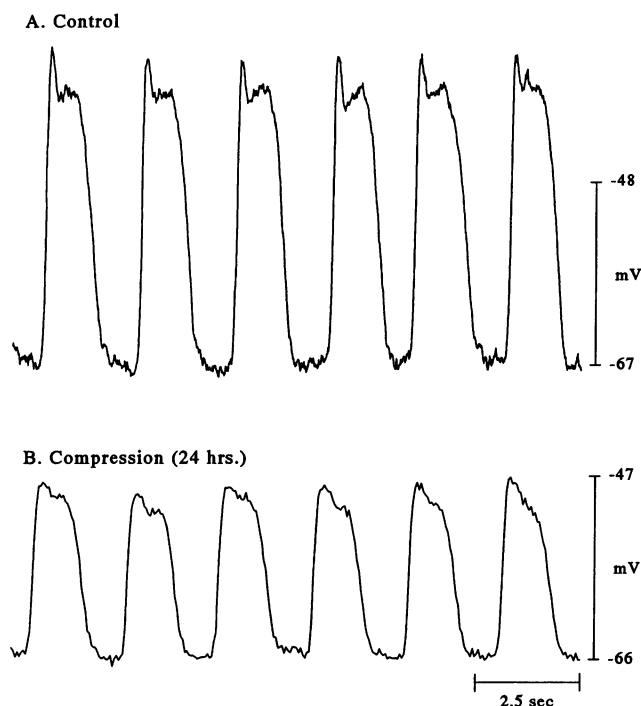
damage, because immediate histologic changes were limited to the mucosa. One week after compression (group VI), mechanical activity was still reduced, but the decrease was not statistically significant (group VI, 0.44 ± 0.102; p = 0.06) compared with controls.

Intracellular electrophysiologic recordings from jejunal circular smooth muscle cells showed that electromechanical activity was altered by surgical compression of the gut. Control jejunal smooth muscle had a resting membrane potential of −69 ± 1.9 mV and spontaneously generated slow waves (29 ± 1.02 events/min) that had the typical configuration of an upstroke (21 ± 2.2 mV) and plateau components (17 ± 2.3 mV) recorded from other gastrointestinal smooth muscles (Fig. 3).[26] Twenty-four hours after compression of the small bowel, the slow wave upstroke and plateau components were significantly decreased (13 ± 0.1 and 12 ± 0.2 mV, respectively). Although slow wave duration tended to be less, together these changes in slow wave configuration resulted in a significant decrease in integrated slow wave area (13.04 ± 0.588 vs. 9.23 ± 0.263 mV/sec) (see Fig. 3). Interestingly, smooth muscle cell resting membrane potentials were not significantly different between controls and compression groups (−69.4 ± 1.9 and −65.0 ± 2.2, respectively). Also, slow wave frequency was not altered by compression (31.2 ± 0.66 events/min) compared with controls.

A. Control

B. Compression (24 hrs.)

Figure 3. Spontaneous intracellular electrical recordings from circular muscle cells in preparations taken from typical control and compressed animals. Panel A shows control intracellular electrical membrane activity. In contrast, Panel B shows that compression caused a marked change in slow wave configuration 24 hours after the surgical procedure.

Morphologic Studies

Histopathologic changes of the intestinal wall were quantitatively graded in all five groups of animals studied on POD 1. Table 1 illustrates the increasing degrees of morphologic change as a function of surgical manipulation. The most extensive degree of damage occurred in the compression group (Fig. 4). This was characterized by massive mucosal sloughing, the loss of the normal mucosal archi-
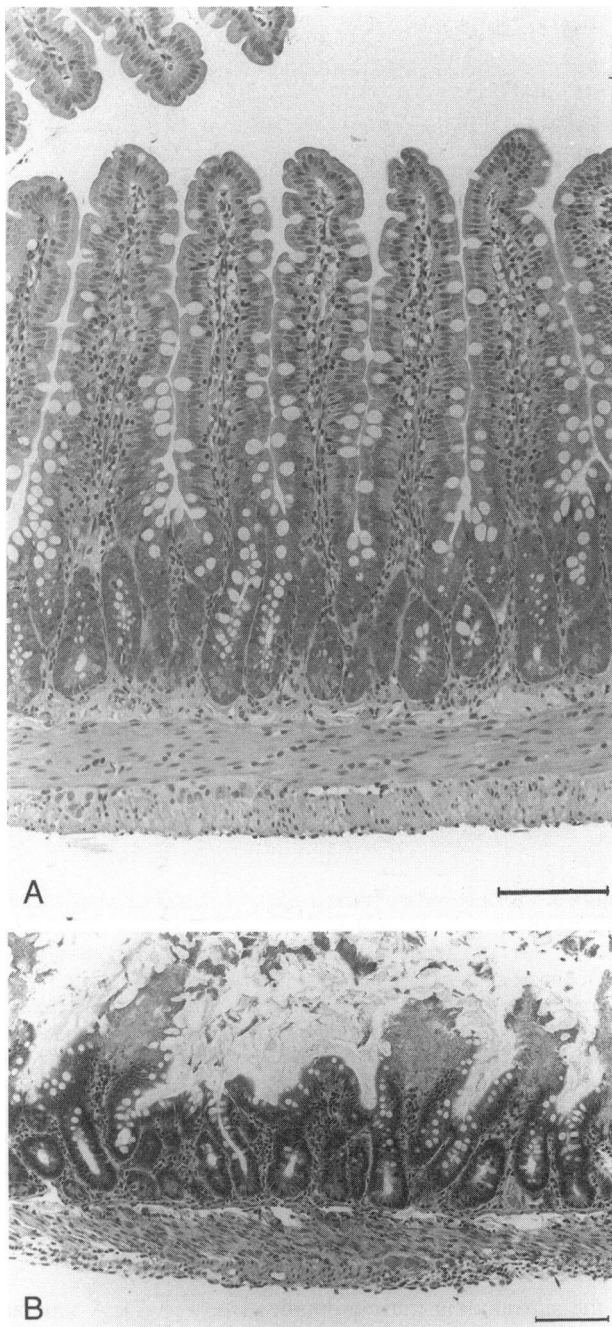
Figure 4. Histology of rat jejunum after surgical manipulation of the gut. Panel A shows the normal architecture in a control rat (grade 0). Panel B demonstrates the structural damage after surgical compression on postoperative day 1 (grade 3). Photomicrographs were taken at a magnification of ×125 (bar = 100 μm). Hematoxylin and eosin stain.

tecture, and various degrees of inflammatory changes throughout the bowel wall. In contrast, the compression group immediately after manipulation was graded at 1 ± 0.41. This group's value of immediate morphologic change was significantly different compared with controls (p = 0.013). However, immediate structural damage was never seen at the level of the muscularis in any of the compressed animals. Further, the level of damage observed on POD 1

was significantly worse than the level observed in group V. Thus, the greater levels of damage observed on POD 1 indicate that most of the histopathologic changes occurred in response to the initiation of an inflammatory cascade and were not the result of the direct physical damage caused by manipulation. Histologically, 7 days after compression (group VI), the muscularis showed a marked improvement, although notable changes still existed.

Infiltration With Myeloperoxidase-Positive Cells

Cross-sections stained with hematoxylin and eosin revealed a dense infiltration of the entire bowel wall by leukocytes. The identity of leukocytes in the intestinal muscularis was first investigated using MPO staining of muscularis whole mounts. As previously reported, few PMNs were observed in the muscularis of control animals (Fig. 5A).[27] The number of MPO-positive PMNs was found to increase as a function of the increasing degrees of surgical intervention. They appeared as large, round to oval cells with an intense dark-brown stain (see Fig. 5). The number of extravasated PMNs was significantly increased in all experimental groups compared with controls (Fig. 6). Minimal surgical manipulation (groups I and II) produced a patchy PMN infiltrate. Intestines subjected to running (group III) and compression (group IV) showed a dense, homogeneous distribution of PMNs throughout the muscularis on POD 1 (see Fig. 5B). A preferential infiltration of the myenteric plexus or the submucosal border of the circular muscle was not noted. This neutrophilic extravasation appeared to continue for a prolonged time, because a significant number of MPO-positive cells were observed even on POD 7 (see Fig. 5C). In whole mounts after running and compression on POD 1, PMNs showed signs of degranulation, with darkly stained granules outside the cell membrane and an enhanced brownish halo-like staining pattern surrounding the densely infiltrated areas (see Fig. 5D). The active degranulation of PMNs was no longer observed on POD 7.

In addition to the darkly stained PMNs, a second cell population could be visualized after an extended period of staining. These cells appeared as small, very round leukocytes that stained a light, transparent brown (see Fig. 5E). These cells were identified as monocytes by double-staining using the monocyte marker ED1.

Muscularis specimens were also assayed for MPO activity. MPO activity was significantly increased in the groups with direct manipulation of the small bowel (control, 2.8 ± 0.29; group III, 7.5 ± 0.80; group IV, 13.2 ± 2.90 mU/g), whereas laparotomy (group I) and eventration (group II) of the bowel did not lead to a significant increase.
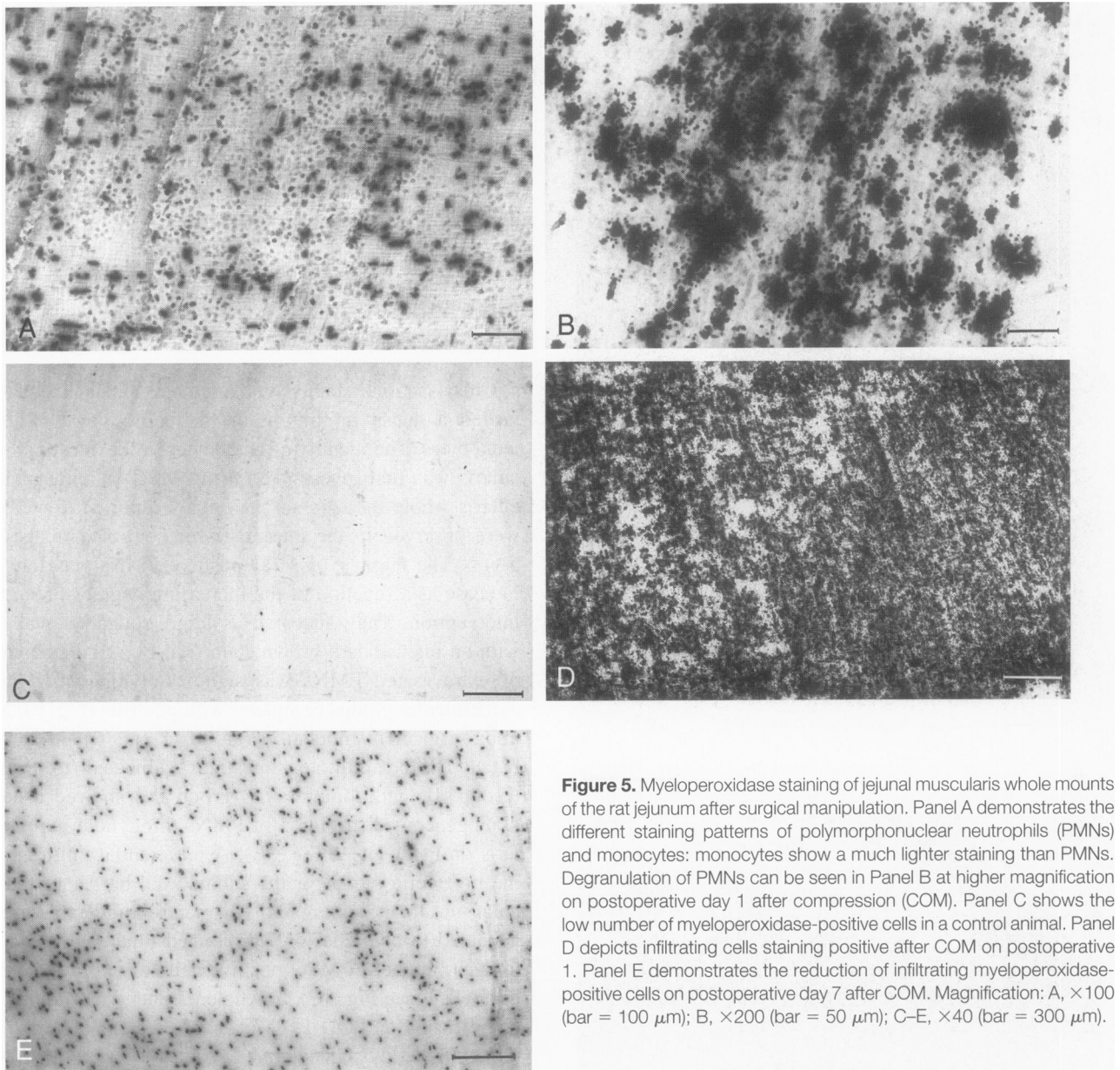
Figure 5. Myeloperoxidase staining of jejunal muscularis whole mounts of the rat jejunum after surgical manipulation. Panel A demonstrates the different staining patterns of polymorphonuclear neutrophils (PMNs) and monocytes: monocytes show a much lighter staining than PMNs. Degranulation of PMNs can be seen in Panel B at higher magnification on postoperative day 1 after compression (COM). Panel C shows the low number of myeloperoxidase-positive cells in a control animal. Panel D depicts infiltrating cells staining positive after COM on postoperative 1. Panel E demonstrates the reduction of infiltrating myeloperoxidase-positive cells on postoperative day 7 after COM. Magnification: A, ×100 (bar = 100 μm); B, ×200 (bar = 50 μm); C–E, ×40 (bar = 300 μm).

Histochemistry and Immunohistochemistry

Immunologic and reparative events after surgery depend on a variety of resident and recruited cells. Histochemical and immunohistochemical methods were used to characterize these leukocytes. In control whole mounts of the intestinal muscularis, ED2-positive cells (resident macrophages) were counted (29 ± 0.95 cells at ×200) and displayed a dense network of dendriform cells with little cytoplasm and several elongated processes (Fig. 7). Differences in the configuration of the macrophages in the resident leukocyte network were seen depending on their locations in the muscularis. In the circular muscle layer, the resident macrophages were squeezed between the muscle bundles and had an elongated, thin shape, oriented in the same direction

as the surrounding muscle bundles (see Fig. 7). A separate and distinct population of macrophages was localized near the myenteric plexus; this population displayed a "fleshy" stellate shape with multiple dendrites and a relatively large central nucleus. The dendrites of these stellate macrophages were not oriented in any particular axial direction. Macrophages in the longitudinal muscle layer were also positioned between the muscle bundles and were fuller and less thinly shaped than the intercalating circular muscle bundle macrophages. Macrophages increased 2.3-fold on POD 1 and 3.1-fold on POD 7 after compression (Fig. 8).

The infiltrating cells were characterized using muscularis whole mounts on POD 1 and 7 after compression. In addition to macrophages, monoclonal antibodies were used to stain monocytes, mast cells, T cells, NK cells, and dendritic
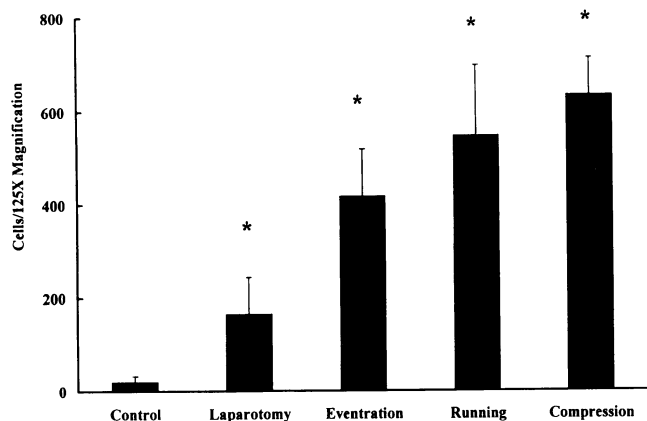
Figure 6. Histogram of infiltrating polymorphonuclear neutrophils in muscularis whole mounts after different degrees of surgical manipulation. Cells were stained using the Hanker-Yates reaction and counted at a magnification of ×125. Data are expressed as mean ± SEM. N = 5 to 7; *, p < 0.05 *vs.* control.

cells. Surface receptor staining was done for MHC class II and the cellular activation marker LFA-1. All cell populations showed a significant increase after POD 1 and 7 (see Fig. 8). On POD 1 after compression (group IV), PMNs, monocytes, and mast cells were the predominant populations and were increased 75.8-, 26.2-, and 66.5-fold, respectively. A structural change in the appearance of the monocytes was noted between day 1 and day 7. The ED1-positive small, round cells demonstrated a gradual maturation into resident macrophages by morphologically progressing through a corniculate shape into a stellate configuration. Immunohistochemically, most of these cells still expressed the ED1 antigen site, and they were counted as monocytes. On POD 7 after compression (group VI), PMNs and monocytes remained elevated 19.5- and 16.4-fold. Mast cells stained with FITC-labeled avidin were also significantly increased after compression on both POD 1 (66.5-fold) and POD 7 (22.0-fold) (see Fig. 8). The identity of the mast cells was also immunohistochemically confirmed using an antibody against mast cell tryptase. Cells of lymphocytic ontogeny (T cells and NK cells), which were infrequently observed in controls, were increased dramatically after compression (T cells 82.6-fold, NK cells 11.6-fold) on POD 1 compared with controls. A significant elevation in these lymphocytes was still present on POD 7 (26.9- and 8.1-fold) (see Fig. 8). B cells could not be demonstrated in the muscularis under control conditions or after compression.

The main antigen-presenting cell in the gastrointestinal tract is the dendritic cell. Dendritic cells in the jejunal muscularis were present under control conditions. After bowel compression, the number of dendritic cells was increased in the muscularis whole mounts by 4.9- and 2.5-fold on POD 1 and 7 (see Fig. 8).

LFA-1, a known cellular activation marker,[28] was barely detectable in normal control specimens. After compression (group IV), nearly all of the counted macrophages expressed this activation marker. LFA-1-positive immunoreactivity

was observed on various leukocyte populations, morphologically identified as macrophages, monocytes, and PMNs. Macrophages are known to be able to process and present antigen to T cells by the expression of class II MHC receptors.[29,30] The percentage of MHC class II-positive macrophages was 64% in control specimens and 100% on POD 1 (group IV) and 83% on POD 7 (group VI) after compression, suggesting a phenotypic change in macrophage function associated with bowel manipulation.
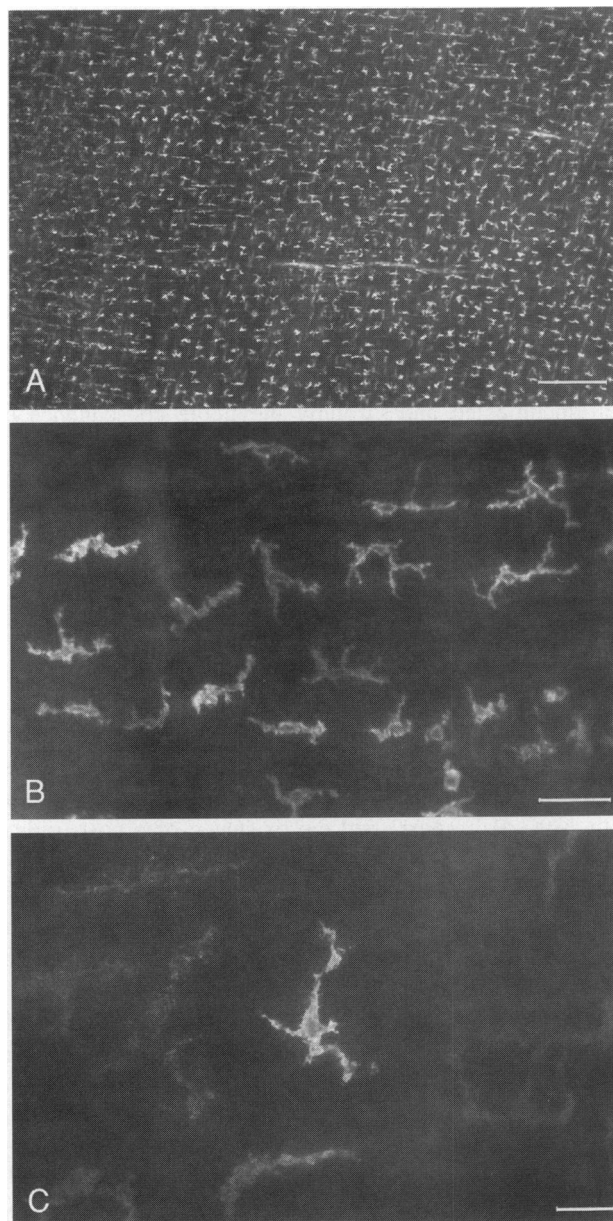

Figure 7. Fluorescence micrograph of a muscularis whole mount from a control rat stained with the mouse antirat ED2 monoclonal antibody, which selectively recognizes a resident macrophage cell membrane epitope. Panels A, B, and C demonstrate the dense homogeneous network of dendritic-appearing macrophages embedded in the muscularis externa of the rat jejunum. Panel A, ×40 (bar = 300 μm); Panel B, ×250 (bar = 50 μm); Panel C, ×400 (bar = 25 μm).
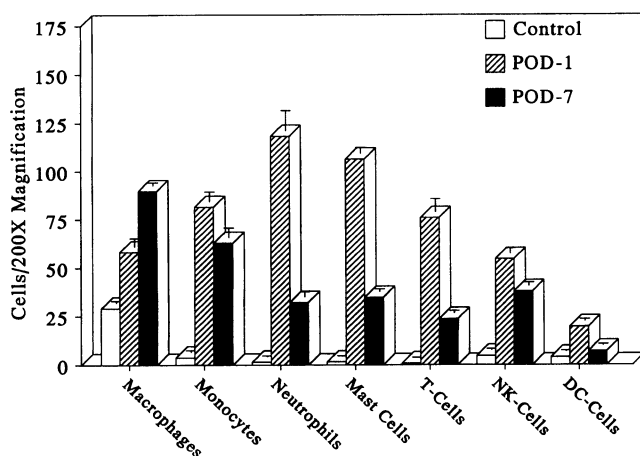
Figure 8. Histogram of the quantification of various infiltrating leukocytes into the intestinal muscularis in control animals and on postoperative days 1 and 7 after compression of the small bowel. Cells were stained using different monoclonal antibodies and counted at a magnification of ×200. Data are expressed as mean ± SEM. N = 5 to 7.

DISCUSSION

Postsurgical ileus, signifying the temporary impairment of coordinated propulsive intestinal peristalsis, remains a well-documented and virtually inevitable consequence of open abdominal surgery. Despite its frequency and impact, accounting for prolonged hospital stays and patient discomfort, little is known of the underlying cellular mechanisms of this surgical conundrum. Our study demonstrates for the first time that clinically relevant and commonly performed surgical manipulation of the gut, but not laparotomy *per se*, results in a failure of gut circular muscle function 24 hours later. Concurrently, the resident network of unactivated muscularis macrophages becomes activated, and by 24 hours there is an extravasation of numerous leukocyte populations into the rat intestinal muscularis, including PMNs, monocytes, mast cells, T cells, NK cells, and dendritic cells.

In 1890, Pal[31] described the existence of a postsurgical reduction in motility. Since then, studies have focused on the duration, intensity, and time course of postsurgical dysmotility in different species using various techniques, including clinical evaluation,[2,32] radiology,[13–15] intraluminal pressure recordings, strain gauges,[5] and *in vivo* myoelectric activity.[4,6,33] The results have been inconsistent.[34,35] Although it intuitively seems reasonable from clinical experience that a more extensive abdominal procedure would cause a more profound ileus, some have concluded that procedure length and the amount of handling did not correlate with the duration of ileus.[4,5] Dahlgren and Selking[15] reported that there is a considerable delay before changes in motility can be observed after extensive surgical procedures in humans. Some of these results appear to disagree with earlier studies and the reality of clinical practice. Taken together, the different results reported in the literature suggest that the data may be explained by differences in species, experimental techniques, type and duration of manip-

ulation, or the time of observation after the surgical procedure.

Five main theories on the mechanism of postsurgical ileus have been proposed. The activation of inhibitory spinal and inhibitory sympathetic reflexes, anesthesia, humoral agents, and inflammation have been implicated as causes.[2]

No prior studies have directly investigated surgically induced immunologic changes in the intestinal muscularis that were correlated with subsequent alterations in gut wall structure and *in vitro* smooth muscle function. Our study in rats shows an association between the extent of surgical manipulation, the degree of cellular infiltration, and inhibition in muscle function. Initially, it would be reasonable to assume that the structural damage and mechanical impairment were directly caused by the manipulation procedure. However, the minimal structural changes observed immediately were associated with a modest decrease in motility. In contrast, the level of structural damage was significantly greater after the subsequent recruitment of leukocytes in all but the group I animals, which underwent laparotomy without gut manipulation.

In our previous investigations, we have found the existence of a dense network of resident macrophages in the intestinal muscularis of different species.[21,36] These cells were first described by Taxi in 1965[37] and were further characterized more recently by Faussone-Pellegrini et al.[38] and Mikkelsen.[39] Relying on histochemical and ultrastructural studies, it was concluded that these resident leukocytes are inactive and quiescent. LFA-1 has generally proven to be a good cellular activation marker for tissue macrophages.[28] In a previous study, we demonstrated that these cells, when activated, express LFA-1 by immunohistochemistry in muscularis whole mounts and in cell cultures.[21,22] In the present study, surgical manipulation resulted in a significant increase in the number and intensity of LFA-1-activated resident macrophages. Further, expansion in the number of LFA-1-positive cells was observed, a finding best explained through the recruitment of primed extravasated PMNs and monocytes. Activation of macrophages generally confers an increased phagocytic and antigen-presenting capability, as well as the possible secretion of more than 100 mediators.[28,40] Macrophage-derived proinflammatory cytokines such as tumor necrosis factor-alpha and interleukins 1 and 6 are known to upregulate adhesion molecules and promote leukocyte recruitment.[41,42] Activation of resident macrophages in other organ systems has also been shown to result in the release and synthesis of a multitude of substances, including arachidonic acid metabolites, reactive oxygen intermediates, NO, hormones, and cytokines.[40] Some of these substances are well-known modulators of smooth muscle activity.[43–45] In a study in rabbits and humans, we were able to demonstrate the capability of N-formyl-MET-LEU-PHE–activated muscularis macrophages to inhibit jejunal circular contractile activity through the release of prostanoids.[36] Activation of the resident muscularis macrophages after surgical manipulation of the intes-

tine is consistent with the idea that these cells mediate the upregulation of adhesion molecules and recruitment of various leukocytes through the synthesis and release of proinflammatory cytokines. Further, they could potentially modulate intestinal motility directly through the release of prostaglandins and NO.

PMNs, known to be the primary constituents of the acute inflammatory response, were a major population of recruited cells in the muscularis in all groups on POD 1. Their activation and recruitment can be influenced by a litany of chemoattractants, including bacterial products, complement, and cytokines.[46] Arriving at their designated site, they act as the first recruited wave of defense against invading pathogens. PMNs secrete numerous potent toxins that are capable of destroying these pathogens. Mechanisms of killing involve the release of preformed granule constituents and the generation of reactive oxygen intermediates.[47] PMNs have been demonstrated in the gastrointestinal tract in a number of inflammatory diseases, including inflammatory bowel disease, ischemia, and reperfusion injury, and small bowel transplantation.[46,48,49] Counting the cells in the muscularis whole mounts proved to be a sensitive technique for determining the recruitment of PMNs: even in the laparotomy group, a significant difference in PMNs could be observed compared with controls. The whole mounts enabled us to look at a much bigger muscle area than the normally used cross-sections. Mature PMNs released into the blood have a relatively short half-life of only 7 hours. Also, extravasated PMNs are believed to be active in tissues for 1 to 4 days.[47] Therefore, all the PMNs counted on POD 7 probably represent only the latest wave of recruits in an ongoing but subsiding process.

Recruited ED1-positive monocytes became the dominant cell type on POD 7. The half-life of the rodent peripheral blood monocyte is slightly longer than the PMN, 17 hours under control conditions.[50] Therefore, the extravasated monocytes observed on POD 7 are certainly the result of the continuous recruitment of these cells. Thus, monocyte recruitment appears to be a relatively more sustained response compared with PMNs. Ontologically, the majority of macrophages stem from maturing extravasated monocytes.[41] Therefore, it seems likely that the observed increase in macrophages on both POD 1 and POD 7 can be explained by a rapid phenotypic change in the recruited monocytes. In fact, the numerous round ED1-positive cells could be seen to develop dendritic-like processes on POD 1. Like the resident macrophages, activated monocytes are known to secrete copious amounts of NO[51] and therefore could participate in the suppression of muscle function after manipulation.

In addition to these phagocytes, we were able to demonstrate an increase in the number of mast cells. The activation of mast cells can be initiated by multiple stimuli, including neuropeptides, cytokines, histamine-releasing factors, bacteria, and bowel manipulation.[52–54] Once activated, these cells frequently degranulate and release potent preformed proinflammatory mediators (*e.g.*, tumor necrosis factor-alpha, histamine, proteases) and produce newly synthesized mediators (prostaglandins, platelet activating factor) and a variety of cytokines.[55,56] Mast cell activation can amplify the recruitment of leukocytes through the release of histamine and the upregulation of adhesion molecules.[57,58] The direct modulation of gastrointestinal motility by degranulating mast cells has been demonstrated to be dependent on 5-hydroxytrytamine and prostaglandin release.[59]

Macrophages in the intestinal tract have the ability to act as antigen-presenting cells, although their main task after contact with foreign antigen is phagocytosis to form a resident defensive barrier.[60] Dendritic cells are highly potent antigen-presenting cells that are immunostimulatory to naïve T cells.[60–62] We observed that surgical manipulation resulted in a fivefold increase in the presence of dendritic cells on POD 1. The biologic queues that cause the migration of these cells are poorly understood. The dendritic presentation of antigen generally takes place in local lymphatic tissues (Peyer's patches, mesenteric lymph nodes) after the migration of dendritic cells. Lymphocytes play a key role in the immunologic response to foreign antigen and the elimination of invading microbes or tumor cells. After an unspecific response of the infiltrating phagocytes, lymphocytes account for a specific immunologic response against foreign material. The increased presence of T cells and NK cells in the surgically manipulated intestinal muscularis therefore suggests that the manipulation stimulus is met by a complex immune response involving both nonspecific and specific arms of the immune system.

A tight correlation was observed between the intensity of infiltration and inhibition in circular muscle contractile activity. The bethanechol-stimulated circular muscle dose-response curves demonstrated a progressive decrease in muscle function in response to increasing degrees of surgical manipulation. These results are consistent with those of Bueno et al.,[3] who showed that handling of the small intestine in rats caused an inhibition of electrical spiking activity compared with laparotomy alone. The association that we observed between the increase in extravasated leukocytes and the incremental degrees of surgical manipulation strongly suggests that the infiltrating cells and their secreted products (*e.g.*, reactive oxygen intermediates, proteases, NO, prostaglandins) play a major role in the suppression in circular activity. Thus, our study provides insight into the pathophysiologic mechanisms that play a major role in ileus after abdominal surgery. However, other forms of postsurgical ileus (*e.g.*, extraabdominal surgery and sepsis) are probably caused through other mechanisms.[22]

These data support the hypothesis that abdominal surgery initiates a scenario of inflammatory events that results in the common clinical phenomenon of postsurgical ileus. Chronologically, we have observed that surgical manipulation triggers the activation of the dense network of resident muscularis macrophages. Secondly, a massive and persis-

tent extravasation of various leukocytes occurred into the intestinal muscularis, and this was subsequently associated with a significant decrease in circular muscle contractile activity. It is tempting to speculate that postsurgical ileus is orchestrated by the immunocompetent muscularis macrophage network and that the subsequent secretion/degranulation of recruited leukocytes causes a severe impairment in muscle function.

Acknowledgments

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